TEST REPORT : 219127054-02-00

Date: 31 MAY 2011 Tel: +65 68851323 Fax: +65 67784301

Client's Ref: OE1000076 Email: Yang.LI@tuv-sud-psb.sg

1. GENERAL

1.1 STUDY TITLE

Acute Dermal Toxicity Study of Bio-X Lullaby

1.2 TEST ITEM IDENTIFICATION

Test item name:	Bio-X Lullaby
Lot No:	20110201
Sterilization condition:	Non-sterile
Quantity:	220 ml per bottle, 2 bottles
Date of manufacture:	1 Feb 2011
Expiry date:	1 Feb 2014

1.2.1 Active Ingredients

(Based on Material Handling Form provided by sponsor)

Composition:	Contains Etofenprox
Purity:	2.50%w/w

1.2.2. Physical features/propertie

(Based on Material Handling Form provided by sponsor)

Colour / state:	Milky White Emulsion
Density:	0.997
pH:	5.83
Solubility:	Soluble in water

1.3 REFERENCE ITEM IDENTIFICATION

Nil

PSB Singapore

2. SPONSOR

OKADA ECOTECH PTE LTD
55, Ayer Rajah Crescent
#03-19/23
Singapore 139949

3. TESTING FACILITY, TESTING SITES AND STAFF

3.1 TESTING FACILITY AND TESTING SITES

Chemical and Materials
Testing Services
TÜV SÜD PSB Pte Ltd
No 1 Science Park Drive
Singapore 118221

3.2 STAFF

Study Director	Ms Li Yang
Study Personnel	Dr Li Zhao Hui
	Mr Chong Koon Chiang
	Mr Tang Xiao Hua

The above staffs are located at

Chemical and Materials
Testing Services
TÜV SÜD PSB Pte Ltd
No 1 Science Park Drive
Singapore 118221

4. STUDY SCHEDULE AND GUIDELINES

4.1 STUDY SCHEDULE

Experimental commencement date	25 Apr 2011
Experimental completion date	10 May 2011

4.2 STUDY GUIDELINES

4.2.1 OECD Guideline For Testing of Chemicals 402: Acute Dermal Toxicity, adopted on 24 Feb 1987

4.2.2 Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Second revised edition, 2007. Chapter 3.1 Acute toxicity

PSB Singapore

5. QUALITY ASSURANCE STATEMENT

This study was audited by Quality Assurance personnel of Chemical and Materials Testing Services as follows:

1. The study plan was audited on 21 Apr 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

2. A process-based audit was conducted between 27 Apr 2011 and 10 May 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

3. The raw data and study report were audited on 31 May 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

The final report has been found to reflect the raw data obtained.

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31 May 2011

CHEW WAN YU **DATE**
QUALITY ASSURANCE
CHEMICAL AND MATERIALS
TESTING SERVICES

PSB Singapore

6. STATEMENT OF COMPLIANCE

6.1 The procedure of the study complies with the study plan as agreed by the sponsor. The conclusion of the test report is based on the raw data obtained from the study.

6.2 This study is in compliance with the regulation of National Advisory Committee on Laboratory Animal Research (NACLAR) of Singapore.

6.3 This study is conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Environmental Health and Safety Publications ENV/MC/CHEM(98)17, Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1, OECD Principles of Good Laboratory Practice (as revised in 1997), Paris 1998.

31 May 2010

MS LI YANG
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

DATE

7. MATERIAL AND METHODS

7.1 PRE-TREATMENT OF TEST ITEM

The test item was used directly for dermal administration without pre-treatment.

7.2 PREPARATION OF TEST SUBSTANCE AND NEGATIVE CONTROL

The test item was used for dermal administration directly. So, in this study, the test substance was the test item. No control substance was used in the study.

7.3 TEST ANIMALS

Species	Rats
Strain	Wistar
Microbiological status	Specific Pathogen Free (SPF)
Age	6 to 8 weeks old
Sex	Male, body weight 342 g to 426 g
	Female, body weight 208 g to 242 g
Number	5 Male and 5 Female
Source	National University of Singapore Centre for Animal Resources (CARE) 7 Perahu Road Singapore 718836
Housing Condition	Individual Ventilated Cage System
Temperature	18 - 22^{o}C
Humidity	30 - 70%
Food	PicoLab® Rodent Diet 20 5053
Water	Tap water

7.4 TEST CONDITIONS

7.4.1. Preparation of test animals

7.4.1.1 The test animals were acclimatised for at least 5 days before the test was conducted.

7.4.1.2 Approximately 24 hours before the test, fur in the dorsal area of each animal's trunk was shaved. The shaved area was not less than 10 percent of the body surface.

7.4.1.3 On the dosing day, the animals were weighed prior to dosing. The test substance was administered by topical application on the shaved area of each animal. The dose level was 5000 mg/kg based on the body weight of each animal.

7.4.1.4 The test item was applied uniformly over the shaved area which was approximately 10 percent of the total surface area. The test item was then held in contact with the skin using a gauze patch and occlusive dressing. The exposure was conducted for 24 hours.

7.4.1.5 At the end of exposure period, residual test item was removed and cleaned carefully with water.

7.4.2 Rationale of selection of limit test

The selection of limit test for this study is due to:

a) Animal welfare consideration- fewer animals would be used for limit test than full study;

b) The dermal toxicity of the test item was expected to be around 5000 mg/kg body weight as declared by sponsor.

7.4.3 Administration level at 5000 mg/kg body weight

Administration	Topical application
Dose level	5000 mg/kg body weight based on weight of the test item
Dose Interval	Single dose

The details of dose for each animal are as follows:

Group No	Animal ID No	Dosing date	Amount of test item used for dosing (g)	Dose level (based on weight of the test item, mg/kg) by body weight
Female	219127054-02-00-F1	26 Apr 2011	1.2	5000
	219127054-02-00-F2		1.0	5000
	219127054-02-00-F3		1.2	5000
	219127054-02-00-F4		1.1	5000
	219127054-02-00-F5		1.2	5000

Group No	Animal ID No	Dosing date	Amount of test item used for dosing (g)	Dose level (based on weight of the test item, mg/kg) by body weight
Male	219127054-02-00-M1	26 Apr 2011	2.1	5000
	219127054-02-00-M2		1.7	5000
	219127054-02-00-M3		1.8	5000
	219127054-02-00-M4		2.0	5000
	219127054-02-00-M5		1.8	5000

7.4.4 Feed and water frequency

Feed was given throughout dosing and observation period. Feed was given in the chamber in the cage.

Water was given *ad libitum* during dosing and observation period. Water was given through plastic bottle.

7.4.5 Observation

The observation of adverse effects was conducted in each animal during the first 30 minutes, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter for a total of 14 days.

On the termination day, all the test animals were euthanized by CO_2 inhalation. Gross necropsy was conducted on all test animals.

8. TEST RESULTS

8.1. Dose level of each test animal and adverse effects

Group No	Animal ID	Body weight (g)	Amount of test item used for dosing (g)	Dose level (based on weight of the test item, mg/kg) by body weight	Adverse effects during and after dosing till endpoint date
Female	219127054 -02-00-F1	234	1.2	5000	No adverse effects observed
	219127054 -02-00-F2	208	1.0	5000	No adverse effects observed
	219127054 -02-00-F3	242	1.2	5000	No adverse effects observed
	219127054 -02-00-F4	226	1.1	5000	No adverse effects observed
	219127054 -02-00-F5	240	1.2	5000	No adverse effects observed

Group No	Animal ID	Body weight (g)	Amount of test item used for dosing (g)	Dose level (based on weight of the test item, mg/kg) by body weight	Adverse effects during and after dosing till endpoint date
Male	219127054 -02-00-M1	426	2.1	5000	No adverse effects observed
	219127054 -02-00-M2	342	1.7	5000	No adverse effects observed
	219127054 -02-00-M3	356	1.8	5000	No adverse effects observed
	219127054 -02-00-M4	398	2.0	5000	No adverse effects observed
	219127054 -02-00-M5	368	1.8	5000	No adverse effects observed

8.2 Body weight (bw, in gram) and body weight changes (change, in gram) of each animal

Group No	Animal ID	26-Apr-11 Dosing day (Day 0)	03-May-11 (Day 7)		10-May-11 (Day 14, Termination Day)	
			Body weight	Change	Body weight	Change
Female	219127054-02-00-F1	234.0	250.5	+16.5	271.2	+20.7
	219127054-02-00-F2	208.1	211.7	+3.6	234.4	+22.7
	219127054-02-00-F3	242.3	260.3	+18.0	274.3	+14.0
	219127054-02-00-F4	226.1	242.1	+16.0	254.7	+12.6
	219127054-02-00-F5	240.0	249.6	+9.6	257.6	+8.0

Group No	Animal ID	26-Apr-11 Dosing day (Day 0)	03-May-11 (Day 7)		10-May-11 (Day 14, Termination Day)	
			Body weight	Change	Body weight	Change
Female	219127054-02-00-M1	426.1	417.9	-8.2	392.1	-25.8
	219127054-02-00-M2	342.2	380.1	+37.9	412.0	+31.9
	219127054-02-00-M3	356.0	377.2	+21.2	414.1	+36.9
	219127054-02-00-M4	398.1	402.5	+4.4	432.0	+29.5
	219127054-02-00-M5	368.0	378.0	+10.0	402.6	+24.6

8.3. Death prior to endpoint

No animal died during dosing and observation period.

8.4. Onset of toxicity and reversal

No adverse effect was observed in all animals during dosing and observation period.

8.5. Necropsy findings

Necropsy was conducted on all the test animals on the termination day. No abnormality was observed on all the test animals.

9. DISCUSSION

Based on the above study,

a) No animal died during dosing and observation period.

b) No adverse effects observed on the test animals during dosing and observation period..

c) Necropsy findings was normal on all animals.

Hence, based on Global Harmonised Classification System (GHS) for acute toxicity hazard categories, LD_{50} cut-off value of Bio-X Lullaby, Lot No. 20110201 is more than 5000 mg/kg body weight.

10. CONCLUSION

Based on the above results and Global Harmonised Classification System (GHS) for acute toxicity hazard categories, the acute dermal toxicity of the test item- Bio-X Lullaby Lot No: 20110201 as Category 5 or unclassified; the LD_{50} value of Bio-X Lullaby Lot No: 20110201 is more than 5000 mg/kg body weight.

11. DEVIATIONS OF THE STUDY PROCEDURES FROM THE STUDY PROTOCOL

11.1 The body weight of the animals (male and female) used for the study are 208 to 426g instead of 200-300g stated in the study plan. The deviation is due to the animals supplied by local supplier. The deviation does not impact the validity of the study.

11.2 The animals used in the study were 6-8 weeks old instead of 8 -12 weeks old stated in the study plan. The deviation is due to the animals supplied by local supplier. The deviation does not impact the validity of the study.

11.3 The test item was applied directly on the shaved area of the animals, not moistened with water for injection as stated in the study plan. The deviation is due to the typo error in the study plan. The deviation does not impact the validity of the study.

12. ARCHIVAL

The study protocol, study plan, study schedule, all the raw data of experiment, audit report of quality assurance unit and other related documentations and the final test report are stored in the archive of TÜV SÜD PSB Pte Ltd.

REMARKS:

1. The above test results relate to the sample of test item as received.

MR TANG XIAO HUA
STUDY PERSONNEL
CHEMICAL AND MATERIALS
TESTING SERVICES

MS LI YANG
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

PSB Singapore

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March 2010